09/373,984

WEST

Generate Collection

Search Results - Record(s) 1 through 1 of 1 returned.

☐ 1. Document ID: US 5620852 A

L7: Entry 1 of 1 File: USPT Apr 15, 1997

US-PAT-NO: 5620852
DOCUMENT-IDENTIFIER: US 5620852 A

TITLE: Nucleic acid preparation methods

DATE-ISSUED: April 15, 1997

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Lin; Lily	Berkeley	CA	N/A	N/A
Cimino; George	Richmond	CA	N/A	N/A
Zhu; Yu S.	Richmond	CA	N/A	N/A

US-CL-CURRENT: 435/6; 435/91.1, 536/22.1, 536/25.3

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw Desc | Image

Generate Collection

Term	Documents
LIGASE$	0
LIGASE.DWPI,EPAB,JPAB,USPT.	10188
LIGASEAND.DWPI,EPAB,JPAB,USPT.	1
LIGASECATALYZED.DWPI,EPAB,JPAB,USPT.	2
LIGASED.DWPI,EPAB,JPAB,USPT.	11
LIGASEL.DWPI,EPAB,JPAB,USPT.	2
LIGASELYSYL.DWPI,EPAB,JPAB,USPT.	1
LIGASEPARSONS.DWPI,EPAB,JPAB,USPT.	1
LIGASEREACTION.DWPI,EPAB,JPAB,USPT.	2
LIGASES.DWPI,EPAB,JPAB,USPT.	1138
(L6 AND LIGASE$).USPT,JPAB,EPAB,DWPI.	1

There are more results than shown above. Click here to view the entire set.

Display 10 Documents, starting with Document: 1

Display Format: CIT Change Format

WEST

End of Result Set

Generate Collection

L7: Entry 1 of 1 File: USPT Apr 15, 1997

DOCUMENT-IDENTIFIER: US 5620852 A
TITLE: Nucleic acid preparation methods

BSPR:
Where purification of RNA is for the purpose of producing template for amplification, it is important to consider the source (i.e. bone marrow, spinal fluid, urine, feces, etc.) and potential polymerase inhibitors that are constituents in such sources. One class of constituents known to inhibit nucleic acid associated enzymes are the "hemes" which include hemin and hematin. Hemin has been reported to inhibit virion-associated reverse transcriptase (RTase) of murine leukemia virus (MuLV) (Tsutsui and Mueller, BBRC 149: 628-634, 1987), DNA ligase (Scher et al., Can. Res. 48: 6278-6284, 1988), cytoplasmic DNA polymerase (Byrnes et al., Biochem. 14, 796-799, 1975), Taq polymerase (PCR Technology, H. A. Erlich (ed.) Stockton Press (1989) p.33), and other enzymatic systems that utilize ATP as a cofactor such as the hemin-controlled protein kinase that affects protein synthesis (Hronis and Traugh, J. Biol. Chem. 261, 6234-6238, 1986), the ATP-dependent ubiquitin-dependent protease pathway (Hershko et al., PNAS USA 81, 1619-1623, 1984), and the ATP-dependent ubiquitin-independent protease pathway (Waxman et al., J. Biol. Chem. 260, 11994-12000, 1985).

BSPR:
Experiments with DNA ligase indicate that hemin at 4 .mu.M or less does not affect DNA ligase activity or DNA substrate integrity. Scher et al., Can. Res. 48, 6278-6284, 1988. Preincubations of DNA ligase with hemin led to half-maximal inhibition of DNA ligase at hemin concentrations of 25-100 .mu.M (depending on the source of the DNA ligase). NAD-dependent DNA ligase from E. coli was not inhibited by hemin at concentrations up to 150 .mu.M. The inhibition of T4 DNA ligase activity and DNA ligase from mouse erythroleukemia (MEL) cells was not reversible by dilution, dialysis, or sucrose gradient centrifugation of cell-free extracts. Incubation of DNA ligase from MEL cells with hemoglobin was not inhibitory.

BSPR:
Binding assays demonstrate that hemin prevents association and causes dissociation of the DNA-cytoplasmic DNA polymerase complex. Hemin at a concentration of 12 .mu.M or higher completely inhibits the formation of DNA-enzyme complex. Byrnes et al., Biochem. 14, 796-799, 1975. This report also shows that hemin inhibition of DNA synthesis is competitive with respect to template and noncompetitive with respect to substrate. Furthermore, inhibition could be reversed by either 1) addition of globin to the polymerase-containing reaction mixture prior to the addition of hemin to the reaction mixture, or 2) addition of globin to hemin followed by addition of this mixture to the polymerase-containing reaction mixture. Inhibition could not be reversed by the addition of globin after introduction of hemin to the polymerase-containing reaction mixture

BSPR:
Experiments with purified hematin and related compounds have shown that they are potent inhibitors of Taq polymerase. Taq polymerase is used in the amplification procedure described by K. B. Mullis et. al., U.S. Pat. Nos. 4,683,195 and 4,683,202. This amplification procedure is a method for increasing the concentration of a segment of a target sequence in a mixture of genomic DNA without cloning or purification. This process for amplifying the target sequence consists of introducing a large excess of two oligonucleotide primers to the DNA mixture containing the desired target sequence, followed by a precise sequence of

thermal cycling in the presence of a DNA polymerase. The two primers are complementary to their respective strands of the double stranded target sequence. To effect amplification, the mixture is denatured and the primers then annealed to their complementary sequences within the target molecule. Following annealing, the primers are extended with a polymerase so as to form a new pair of complementary strands. The steps of denaturation, primer annealing, and polymerase extension can be repeated many times (i.e. denaturation, annealing and extension constitute one "cycle;" there can be numerous "cycles") to obtain a high concentration of an amplified segment of the desired target sequence. The length of the amplified segment of the desired target sequence is determined by the relative positions of the primers with respect to each other, and therefore, this length is a controllable parameter. By virtue of the repeating aspect of the process, the method is referred to by the inventors as the "Polymerase Chain Reaction" (hereinafter PCR). Because the desired amplified segments of the target sequence become the predominant sequences (in terms of concentration) in the mixture, they are said to be "PCR amplified".

BSPR:
The invention is particularly useful where it is desired to amplify said nucleic acid recovered in step (d). In a preferred embodiment, amplification is performed on recovered ribonucleic acid by adding a thermostable DNA polymerase having endogenous reverse transcriptase activity to said recovered ribonucleic acid. The method is compatible with the use of deoxyribonucleoside triphosphate dUTP (preferrably at approximately 400 mM).

DEPR:
Template specificity is achieved in most amplification techniques by the choice of enzyme. Amplification enzymes are enzymes that, under conditions they are used, will process only specific sequences of nucleic acid in a heterogeneous mixture of nucleic acid. For example, in the case of Q.beta. replicase, MDV-1 RNA is the specific template for the replicase. D. L. Kacian et. al., Proc. Nat. Acad. Sci USA 69:3038 (1972). Other nucleic acid will not be replicated by this amplification enzyme. Similarly, in the case of T7 RNA polymerase, this amplification enzyme has a stringent specificity for its own promoters. M. Chamberlin et al., Nature 228:227 (1970). In the case of T4 DNA ligase, the enzyme will not ligate the two oligonucleotides where there is a mismatch between the oligonucleotide substrate and the template at the ligation junction. D. Y. Wu and R. B. Wallace, Genomics 4:560 (1989). Finally, Taq polymerase, by virtue of its ability to function at high temperature, is found to display high specificity for the sequences bound and thus defined by the primers; the high temperature results in thermodynamic conditions that favor primer hybridization with the target sequences and not hybridization with non-target sequences. PCR Technology, H. A. Erlich (ed.) (Stockton Press 1989).

DEPR:
The present invention offers a radical change from the cumbersome nucleic acid whole blood method of density centrifugation and provides a flexible approach to whole blood processing for nucleic acid amplification (see FIG. 1). Importantly, the method of whole blood processing of the present invention can be carried out with i) only microliter amounts of blood (e.g. fingerstick or heelstick), ii) stored blood (including dried blood), and iii) the same, single reaction vessel used for amplification (allowing for quantitative recovery of cellular nucleic acid, in contrast, to the low-yield density centrifugation method). Because of these features, the method of the present invention is also amenable to automation.

DEPR:
The step of lysing the whole blood for release of RNA for conversion to cDNA is artful. It is important to release the RNA under conditions where it is not degraded due to simultaneous release of nucleases. The protease K must degrade the nucleases faster than the nucleases degrade the RNA, or if not, there will be no "net RNA" left for transcription by RT. In this experiment, free RNA was added to whole blood, and then lysis was carried out. To minimize the RNA loss, RNAsin is added (which is a potent RNAse inhibitor) during the protease K step. Salt (KCl) and DTT concentration are very important. For optimum results the following reactions conditions were found:

DEPR:
Once RNA was recovered, this was amplified (5-10 .mu.l of the resuspension) by a

coupled RT/PCR amplification reaction using a thermostable DNA polymerase having endogenous reverse transcriptase activity ("rTth RT/PCR Kit", Perkin Elmer Cetus, Norwalk, Conn.) and the products detected with ethidium bromide on agarose gels. The procedure for the coupled RT/PCR assay was as follows:

DEPR:
In this example, deoxyribonucleic acid was made from ribonucleic acid which was recovered according to one embodiment of the sample preparation method of the present invention. The method of the present invention was compared as in Example 25 (above) by preparing known HCV plasma samples in parallel with the commercially available IsoQuick protocol (Microprobe Corp., Bothel, Wash.). Once RNA was recovered, this was amplified (5-10 ul of the resuspension) by a coupled RT/PCR amplification reaction using a DNA polymerase with endogenous reverse transciptase activity ("rTth RT/PCR Kit", Perkin Elmer Cetus, Norwalk, Conn.) and the products detected with ethidium bromide on agarose gels. The procedure for the coupled RT/PCR assay was as described in Example 25.

DETL:
_____ H.sub.2 O 6.3 .mu.l 10XRT Rxn. Buffer (100 mM Tris-HCl (pH 8.3), 2.0 .mu.l 900 mM KCl 10 mM MnCl.sub.2 -0.85 mM final concentration 1.7 .mu.l dNTP: 2 mM each dATP, dCTP, dGTP, & dTTP (in 2.0 .mu.l H.sub.2 O, pH 7.0) RT "downstream" primer KY78 (1.5 .mu.M in H.sub.2 O) - 2.0 .mu.l 3 picomole/rxn. PCR "upstream" primer KY80 (1.5 .mu.M in H.sub.2 O) - 2.0 .mu.l 3 picomole/rxn. rTth DNA polymerase: 2.5 units/ul in 2.0 .mu.l 1X Enzyme storage buffer = 20 mM Tris-HCl (pH 7.5), 100 mM KCl, 0.1 mM EDTA, 1 mM DTT, 0.2% Tween (Pierce Surfactants), 50% glycerol (v/v) Template nucleic acid: less than 250 ng total in TE, 2.0 .mu.l 10 mM Tris 1 mM EDTA) Total Reaction volume 20.0 .mu.l _____

CLPR:
10. The method of claim 9 wherein said amplifying of said ribonucleic acid is performed by adding a thermostable DNA polymerase having endogenous reverse transcriptase activity to said recovered ribonucleic acid.

CLPR:
14. The method of claim 7 wherein said amplifying of said ribonucleic acid is performed by adding a thermostable DNA polymerase having endogenous reverse transcriptase activity to said recovered ribonucleic acid.

CLPR:
19. The method of claim 13 wherein said amplifying of said ribonucleic acid is performed by adding a thermostable DNA polymerase having endogenous reverse transcriptase activity to said recovered ribonucleic acid.

09373,284

Trying 3106016892...Open

Welcome to STN International! Enter x:x
LOGINID:ssspta1806jxt
PASSWORD:
TERMINAL (ENTER 1, 2, 3, OR ?):2

* * * * * * * * * * Welcome to STN International * * * * * * * * * *

NEWS 1		Web Page URLs for STN Seminar Schedule - N. America
NEWS 2	Sep 29	The Philippines Inventory of Chemicals and Chemical Substances (PICCS) has been added to CHEMLIST
NEWS 3	Oct 27	New Extraction Code PAX now available in Derwent Files
NEWS 4	Oct 27	SET ABBREVIATIONS and SET PLURALS extended in Derwent World Patents Index files
NEWS 5	Oct 27	Patent Assignee Code Dictionary now available in Derwent Patent Files
NEWS 6	Oct 27	Plasdoc Key Serials Dictionary and Echoing added to Derwent Subscriber Files WPIDS and WPIX
NEWS 7	Nov 29	Derwent announces further increase in updates for DWPI
NEWS 8	Dec 5	French Multi-Disciplinary Database PASCAL Now on STN
NEWS 9	Dec 5	Trademarks on STN - New DEMAS and EUMAS Files
NEWS 10	Dec 15	2001 STN Pricing
NEWS 11	Dec 17	Merged CEABA-VTB for chemical engineering and biotechnology
NEWS 12	Dec 17	Corrosion Abstracts on STN
NEWS 13	Dec 17	SYNTHLINE from Prous Science now available on STN
NEWS 14	Dec 17	The CA Lexicon available in the CAPLUS and CA files
NEWS 15	Jan 05	AIDSLINE is being removed from STN
NEWS 16	Feb 06	Engineering Information Encompass files have new names
NEWS 17	Feb 16	TOXLINE no longer being updated

NEWS EXPRESS	FREE UPGRADE 5.0e FOR STN EXPRESS 5.0 WITH DISCOVER! (WINDOWS) NOW AVAILABLE
NEWS HOURS	STN Operating Hours Plus Help Desk Availability
NEWS INTER	General Internet Information
NEWS LOGIN	Welcome Banner and News Items
NEWS PHONE	Direct Dial and Telecommunication Network Access to STN
NEWS WWW	CAS World Wide Web Site (general information)

Enter NEWS followed by the item number or name to see news on that specific topic.

All use of STN is subject to the provisions of the STN Customer agreement. Please note that this agreement limits use to scientific research. Use for software development or design or implementation of commercial gateways or other similar uses is prohibited and may result in loss of user privileges and other penalties.

* * * * * * * * * * * * * STN Columbus * * * * * * * * * * * * * * * *

FILE 'HOME' ENTERED AT 12:58:59 ON 14 MAR 2001

=> file medline biosis caplus

	SINCE FILE ENTRY	TOTAL SESSION
COST IN U.S. DOLLARS		

FULL ESTIMATED COST 0.15 0.15

FILE 'MEDLINE' ENTERED AT 12:59:34 ON 14 MAR 2001

FILE 'BIOSIS' ENTERED AT 12:59:34 ON 14 MAR 2001
COPYRIGHT (C) 2001 BIOSIS(R)

FILE 'CAPLUS' ENTERED AT 12:59:34 ON 14 MAR 2001
USE IS SUBJECT TO THE TERMS OF YOUR STN CUSTOMER AGREEMENT.
PLEASE SEE "HELP USAGETERMS" FOR DETAILS.
COPYRIGHT (C) 2001 AMERICAN CHEMICAL SOCIETY (ACS)

=> s (single reaction or single vessel or one vessel)(10a)DNA polymerase#(10a)RNA polymerase#(10a)reverse transcriptase#(10a)ligase

L1 0 (SINGLE REACTION OR SINGLE VESSEL OR ONE VESSEL)(10A) DNA POLYME
RASE#(10A) RNA POLYMERASE#(10A) REVERSE TRANSCRIPTASE#(10A) LIGASE

=> (single reaction or single vessel or one vessel)(10a)DNA polymerase#(10a)RNA polymerase#(10a)reverse transcriptase#(10a)RNase

(SINGLE IS NOT A RECOGNIZED COMMAND
The previous command name entered was not recognized by the system.
For a list of commands available to you in the current file, enter
"HELP COMMANDS" at an arrow prompt (=>).

=> s (single reaction or single vessel or one vessel)(10a)DNA polymerase#(10a)RNA polymerase#(10a)reverse transcriptase#(10

MISSING OPERATOR 'SCRIPTASE#(10'
The search profile that was entered contains terms or
nested terms that are not separated by a logical operator.

=> s single reaction or single vessel or one vessel

L2 5858 SINGLE REACTION OR SINGLE VESSEL OR ONE VESSEL

=> s l2 and DNA polymerase# and RNA polymerase# and reverse transcriptase# and RNAse#

L3 2 L2 AND DNA POLYMERASE# AND RNA POLYMERASE# AND REVERSE TRANSCRIP
TASE# AND RNASE#

=> d l3 1-2 bib ab

L3 ANSWER 1 OF 2 CAPLUS COPYRIGHT 2001 ACS
AN 1998:344534 CAPLUS
DN 129:13211
TI Method and kit for direct isothermal sequencing of nucleic acids
IN Dunn, James M.; Digby, Thomas J.
PA Visible Genetics Inc., Can.; Dunn, James M.; Digby, Thomas J.
SO PCT Int. Appl., 22 pp.
CODEN: PIXXD2
DT Patent
LA English
FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 9821361	A1	19980522	WO 1997-CA848	19971112

W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, HU, ID, IL, IS, JP, KE, KG, KP, KR,

KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
RW: GH, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG

AU 9749384 A1 19980603 AU 1997-49384 19971112
PRAI US 1996-31257 19961112
WO 1997-CA848 19971112

AB Direct detn. of the sequence of an RNA sample is performed under isothermal conditions. An RNA sample contg. a target nucleic acid is combined in a **single reaction** vessel with a reaction mixt. contg. two polynucleotide primers, a first primer that specifically hybridizes with a target sequence near the 3' end of an antisense copy of the target nucleic acid. At least one of the primers is labeled with a detectable label, and at least one of the first or second primers has an **RNA polymerase** transcription initiation signal at its 5' end, which signal does not specifically hybridize to the RNA target. The reaction mixt. also contains ribonucleotide triphosphates for RNA synthesis, deoxyribonucleotide triphosphates for DNA synthesis, at least one type of dideoxynucleotide triphosphate chain-terminator, and enzymes with the activity of **reverse transcriptase**, **RNAse** H, **RNA Polymerase** and a low discrimination **DNA Polymerase** such as Thermo SequenaseTM. The combined reactants are incubated under isothermal conditions for a length of time sufficient to generate chain-terminated reaction products, and the chain-terminated reaction products are then detected after electrophoretic sepn.

L3 ANSWER 2 OF 2 CAPLUS COPYRIGHT 2001 ACS
AN 1996:458032 CAPLUS
DN 125:107035
TI Terminal repeat amplification method for nucleic acids
IN Sooknanan, Roy; Malek, Lawrence
PA Akzo Nobel N.V., Neth.
SO PCT Int. Appl., 94 pp.
CODEN: PIXXD2
DT Patent
LA English
FAN.CNT 1

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI WO 9617079	A1	19960606	WO 1995-EP4694	19951128
W: AM, AU, BB, BG, BR, BY, CA, CN, CZ, EE, FI, GE, HU, JP, KE, KG, KP, KR, KZ, LK, LR, LT, LV, MD, MG, MN, MW, MX, NO, NZ, PL, RO, RU, SD, SI, SK, TJ, TT, UA, US, UZ, VN				
RW: KE, LS, MW, SD, SZ, UG, AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG				
US 5665545	A	19970909	US 1994-345505	19941128
CA 2205563	AA	19960606	CA 1995-2205563	19951128
AU 9641767	A1	19960619	AU 1996-41767	19951128
AU 711589	B2	19991014		
EP 796343	A1	19970924	EP 1995-940256	19951128
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LI, LU, MC, NL, PT, SE				
JP 10510161	T2	19981006	JP 1995-518184	19951128
FI 9702238	A	19970527	FI 1997-2238	19970527

PRAI US 1994-345505 19941128
WO 1995-EP4694 19951128

AB This invention relates to a process for amplifying a specific nucleic acid sequence or its complement at a relatively const. temp. and without serial addn. of reagents. The process provides in a **single**

reaction medium an **RNA polymerase**, **DNA polymerase**, a **RNase** that hydrolyzes RNA of an RNA-DNA hybrid without hydrolyzing single or double-stranded RNA or DNA, and ribonucleoside and deoxyribonucleoside triphosphates. The process then provides an RNA first template in the reaction medium. The RNA first template comprises a sequence complementary to a specific nucleic acid sequence, minus-sense sequences for a promoter and initiation site that are recognized by the **RNA polymerase**, and a 5'-terminal sequence that is complementary to at least the minus-sense sequence of the initiation site. Thus, the RNA first template has in inverted repeat sequence which could fold into a 5'-terminal stem-loop structure. The **DNA polymerase** uses the RNA first template to synthesize a DNA second template that together comprise an RNA-DNA hybrid. The DNA second template has plus-sense sequences of the promoter and the initiation site, and a 3'-terminal priming sequence that is complementary to the plus-sense sequence of the initiation site. The **RNase** then hydrolyzes an RNA which comprises the RNA-DNA hybrid, allowing the 3'-terminal priming sequence to hybridize to the plus-sense sequence of the initiation site in the DNA second template. The **DNA polymerase** then uses the DNA second template to synthesize the promoter by extending the 3'-terminal priming sequence of the DNA second template. The resulting partially double-stranded DNA has a promoter oriented toward the apex of a stem-loop structure. The **RNA polymerase** then recognizes the promoter and transcribes the DNA second template, thereby providing copies of the RNA first template. The process thereafter maintains the reaction conditions for a time sufficient to achieve a desired amplification of the specific nucleic acid sequence or its complement. This invention includes a kit contg. the reagents of this invention.

L5 ANSWER 1 OF 1 CAPLUS COPYRIGHT 2001 ACS
AN 1996:458032 CAPLUS
DN 125:107035
TI Terminal repeat amplification method for nucleic acids
IN Sooknanan, Roy; Malek, Lawrence
PA Akzo Nobel N.V., Neth.
SO PCT Int. Appl., 94 pp.
CODEN: PIXXD2
DT Patent
LA English
FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 9617079	A1	19960606	WO 1995-EP4694	19951128
	W: AM, AU, BB, BG, BR, BY, CA, CN, CZ, EE, FI, GE, HU, JP, KE, KG, KP, KR, KZ, LK, LR, LT, LV, MD, MG, MN, MW, MX, NO, NZ, PL, RO, RU, SD, SI, SK, TJ, TT, UA, US, UZ, VN				
	RW: KE, LS, MW, SD, SZ, UG, AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG				
	US 5665545	A	19970909	US 1994-345505	19941128
	CA 2205563	AA	19960606	CA 1995-2205563	19951128
	AU 9641767	A1	19960619	AU 1996-41767	19951128
	AU 711589	B2	19991014		
	EP 796343	A1	19970924	EP 1995-940256	19951128
	R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LI, LU, MC, NL, PT, SE				
	JP 10510161	T2	19981006	JP 1995-518184	19951128
	FI 9702238	A	19970527	FI 1997-2238	19970527
PRAI	US 1994-345505	19941128			
	WO 1995-EP4694	19951128			

AB This invention relates to a process for amplifying a specific nucleic acid sequence or its complement at a relatively const. temp. and without serial addn. of reagents. The process provides in a **single reaction** medium an **RNA polymerase**, **DNA polymerase**, a **RNase** that hydrolyzes RNA of an RNA-DNA hybrid without hydrolyzing single or double-stranded RNA or DNA, and ribonucleoside and deoxyribonucleoside triphosphates. The process then provides an RNA first template in the reaction medium. The RNA first template comprises a sequence complementary to a specific nucleic acid sequence, minus-sense sequences for a promoter and initiation site that are recognized by the **RNA polymerase**, and a 5'-terminal sequence that is complementary to at least the minus-sense sequence of the initiation site. Thus, the RNA first template has in inverted repeat sequence which could fold into a 5'-terminal stem-loop structure. The **DNA polymerase** uses the RNA first template to synthesize a DNA second template that together comprise an RNA-DNA hybrid. The DNA second template has plus-sense sequences of the promoter and the initiation site, and a 3'-terminal priming sequence that is complementary to the plus-sense sequence of the initiation site. The **RNase** then hydrolyzes an RNA which comprises the RNA-DNA hybrid, allowing the 3'-terminal priming sequence to hybridize to the plus-sense sequence of the initiation site in the DNA second template. The **DNA polymerase** then uses the DNA second template to synthesize the promoter by extending the 3'-terminal priming sequence of the DNA second template. The resulting partially double-stranded DNA has a promoter oriented toward the apex of a stem-loop structure. The

RNA polymerase then recognizes the promoter and transcribes the DNA second template, thereby providing copies of the RNA first template. The process thereafter maintains the reaction conditions for a time sufficient to achieve a desired amplification of the specific nucleic acid sequence or its complement. This invention includes a kit contg. the reagents of this invention.

AB This invention relates to a process for amplifying a specific nucleic acid sequence or its complement at a relatively const. temp. and without serial addn. of reagents. The process provides in a **single reaction** medium an **RNA polymerase**, **DNA polymerase**, a **RNase** that hydrolyzes RNA of an RNA-DNA hybrid without hydrolyzing single or double-stranded RNA or DNA, and ribonucleoside and deoxyribonucleoside triphosphates. The process then provides an RNA first template in the reaction medium. The RNA first template comprises a sequence complementary to a specific nucleic acid sequence, minus-sense sequences for a promoter and initiation site that are recognized by the **RNA polymerase**, and a 5'-terminal sequence that is complementary to at least the minus-sense sequence of the initiation site. Thus, the RNA first template has in inverted repeat sequence which could fold into a 5'-terminal stem-loop structure. The **DNA polymerase** uses the RNA first template to synthesize a DNA second template that together comprise an RNA-DNA hybrid. The DNA second template has plus-sense sequences of the promoter and the initiation site, and a 3'-terminal priming sequence that is complementary to the plus-sense sequence of the initiation site. The **RNase** then hydrolyzes an RNA which comprises the RNA-DNA hybrid, allowing the 3'-terminal priming sequence to hybridize to the plus-sense sequence of the initiation site in the DNA second template. The **DNA polymerase** then uses the DNA second template to synthesize the promoter by extending the 3'-terminal priming sequence of the DNA second template. The resulting partially double-stranded DNA has a promoter oriented toward the apex of a stem-loop structure. The **RNA polymerase** then recognizes the promoter and transcribes the DNA second template, thereby providing copies of the RNA first template. The process thereafter maintains the reaction conditions for a time sufficient to achieve a desired amplification of the specific nucleic acid sequence or its complement. This invention includes a kit contg. the reagents of this invention.

IT Virus, animal
(Moloney murine leukemia, **reverse transcriptase** for use in TRAM technique from; terminal repeat amplification method for nucleic acids)

IT Virus, bacterial
(SP6, **RNA polymerase** for use in TRAM technique from; terminal repeat amplification method for nucleic acids)

IT Virus, bacterial
(T3, **RNA polymerase** for use in TRAM technique from; terminal repeat amplification method for nucleic acids)

IT Virus, bacterial
(T7, **RNA polymerase** for use in TRAM technique from; terminal repeat amplification method for nucleic acids)

IT Virus, animal
(avian myeloblastosis, **reverse transcriptase** for use in TRAM technique from; terminal repeat amplification method for nucleic acids)

IT Virus, bacterial
(gh-1, **RNA polymerase** for use in TRAM technique from; terminal repeat amplification method for nucleic acids)

IT Virus, bacterial
(phi II, **RNA polymerase** for use in TRAM technique from; terminal repeat amplification method for nucleic acids)

IT Virus, animal
(retro-, **reverse transcriptase** for use in TRAM

technique from; terminal repeat amplification method for nucleic acids)

IT 9014-24-8, **RNA polymerase** 9015-85-4, DNA **ligase** 9050-76-4, **RNase** H 9068-38-6, **Reverse transcriptase**
RL: BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)
(terminal repeat amplification method for nucleic acids)